Journal of Forestry Research (2011) 22(4): 507–517
DOI 10.1007/s11676-011-0171-y

ORIGINAL PAPER

Genetic diversity and relationship between cultivated clones of *Dalbergia sissoo* of wide geographical origin using RAPD markers

H. S. Ginwal • S. S. Maurya • P. Chauhan

Received: 2010-02-10; Accepted: 2010-05-12

Abstract: Random Amplified Polymorphic DNA (RAPD) polymorphism was employed to assess the genetic diversity in the elite germplasm of *Dalbergia sissoo*. Sixty-seven clones that are under cultivation in northern India, originated from six different states of India and Nepal were analyzed with 30 RAPD primers that generated a total of 342 fragments out of which 290 were polymorphic. Total genetic diversity (*H*t) varied between 0.01 and 0.37, with an average of 0.19. Shannon's Information index (I) varied between 0.02 and 0.54, with an average of 0.31. Marker attributes like Polymorphism Information Content (PIC), Marker Index (MI) and Effective Multiplex Ratio (EMR) values were calculated to assess the discriminatory power of 30 primers used. The PIC values ranged from 0.01 to 0.37 with an average of 0.17 per primer and the EMR ranged from 0.17 to 21.00 with a mean of 8.66 across all genotypes. Closely related clones were C49 and C51 with similarity index of 0.86 while the least similar or most dissimilar clones were C14 and S-DB showing similarity index of 0.58. The UPGMA-phenogram categorized the 67 clones into six clusters based on genetic similarity and dissimilarity. The clustering of clones in relation to their geographical location has been discussed.

Keywords: clones; *Dalbergia sissoo*; genetic diversity; polymorphic; RAPD

Introduction

Dalbergia sissoo Roxb. known as Shisham is a popular tropical timber tree species native to foothills of Himalayas of India, Pakistan, Nepal and Indo-Gangetic basin. It grows in the entire sub-Himalayan tract and also in the Himalayan valley up to an elevation of about 1 500 m along streams on alluvial soils. The species has great value for timber and is very important raw material for wood based industries, especially building construction and furniture because of strength, pleasant brown colour and beautiful grains. It is also used for shelterbelts and fuel wood in the sub-humid and drier areas. Being an important tropical timber tree species, and its popularity due to its fast growth and multiple uses, *D. sissoo* is raised for rural as well as industrial plantations.

The natural *D. sissoo* forests have come under considerable pressure from human disturbance. In last few years the tree species is suffering from wilt disease resulting in large-scale mortality, mostly in plantations. The disease is now commonly found throughout the natural distribution area in South Asia and is causing widespread death of shisham forests. One of the important reasons of mortality appears to be the promotion of monoculture plantations with narrow genetic base. The mortality is also prevalent in areas where no attention is paid to the quality of the planting material and the site selection. Seeds used in plantations rarely come from a broad genetic pool. It has been estimated that around 90% of the shisham plantations in terai region of Nepal have been suffering from this diseases (Parajuli et al. 1999).

In India, the comprehensive breeding programme for *D. sissoo* started since 1993. Countrywide surveys including Nepal for identifying superior phenotypes (plus trees) of *D. sissoo* were carried out. The disease was not serious during the start of the tree improvement programme and hence was not given high priority during selection. Seeds and root suckers from the selected phenotypes were used for the establishment of seed orchards. More than 400 superior phenotypes were available for collection of seeds and their seed orchards were established in north Indian states of Haryana, Punjab, Uttarakhand and Uttar Pradesh. Besides, the selected phenotypes were clonally propagated and deployed under clonal tests in different locations. Through this process more than 100 good performing clones in respect of growth and survival were identified. Presently these clones are being propagated on large-scale for their cultivation under clonal forestry. There were likely chances that the many of

The online version is available at http://www.springerlink.com

H. S. Ginwal (✉) • Shalini Singh Maurya • Priti Chauhan
Division of Genetics and Tree Propagation, Forest Research Institute, P.O. I.P.E Kaulagarh Road, Dehradun 248195 (Uttarakhand) India.
Email: ginwalhs@icfre.org; ginwalhs@rediffmail.com

Responsible editor: Hu Yanbo

the selected clones were closely related. Hence, identification of genetically divergent clones, their deployment and maintaining sufficient genetic variability in the shisham plantations are the important steps to combat shisham mortality.

To date, no universal marker system is available for codominant nuclear loci in *D. sissoo*. The unavailability of codominant markers has lead to the use of Random Amplified Polymorphic DNA (RAPD) markers (Williams et al. 1990) for genetic analysis of shisham clones. RAPD and ISSR (Inter Simple Sequence Repeat) markers have been used in many population genetic studies and in detecting clonal diversity (Parsons et al. 1997; Esselman et al. 1999; Li and Ge 2001). The technique has been successfully used to study genetic diversity in many plant genera such as mango (Schnell et al. 1995), Eucalyptus (Keil and Griffin 1994; Ginwal and Maurya 2008), Himalayan poplar (Rajgopal et al. 2000), Cacao (Whitkus et al. 1998), Spruce (Scheepers et al. 1997), Populus spp. (Castiglione et al. 1993). In a recent publication, RAPD markers have been found more informative than ISSRs in the assessment of genetic diversity in shisham (Arif et al. 2009).

Keeping in view the above, the present investigation was conducted to analyze genetic diversity and establish genetic relationship between the selected 67 clones of *D. sissoo* that are under large-scale propagation and cultivation in northern India. The aim is also to provide insight information on genetic diversity status in superior germplasm of *D. sissoo* in India, which will enable to compare it with other tropical tree species for developing a long term breeding strategy and providing a scientific basis for the management of genetic resources of *D. sissoo*. The investigation will help to identify genetically divergent clones for their deployment under clonal forestry programs to optimize genetic gains.

Materials and methods

Plant material

Sixty-seven clones of *D. sissoo*, which are under cultivation in north India, were used for the present study. The clones originate from the diverse geographical region from Haryana, Punjab, Rajasthan, Uttarakhand, Uttar Pradesh and Nepal. The list of genotypes with their source is presented in Table 1. Young foliages from ramets of 67 clones established in a vegetative multiplication garden of Forest Research Institute, Dehradun, India were collected, tagged and stored in deep freezer at -80°C to prevent degradation of DNA in the tissues.

Isolation of DNA

A total of 500 mg young leaves without mid rib, veins and margins were chopped and ground to fine powder using liquid nitrogen in a pre-chilled mortar and pestle. A protocol for *D. sissoo* genomic DNA isolation developed by Ginwal and Maurya (2010) was used for the extraction of the genomic DNA from all the samples representing 67 clones of shisham.

PCR amplification

A total of 50 primers from the list given by Mosseler et al. (1992) and Operon company (QIAGEN Operon, 1000 Atlantic Avenue, Almeda, CA 94501, USA) were initially used in the present study, of which 30 primers (Mosseler-29, Mosseler-31, Mosseler-33, Mosseler-119, Mosseler-122, Mosseler-132, Mosseler-147, Mosseler-156, Mosseler-182, Mosseler-186, Mosseler-188, Mosseler-198, Mosseler-199, OPA-02, OPA-04, OPA-05, OPA-06, OPA-07, OPA-08, OPA-09, OPA-10, OPA-11, OPA-14, OPA-16, OPA-17, OPA-18, OPA-20, OPAF-07, OPAF-16, OPG-09) were selected based upon their reproducibility and polymorphism for RAPD fingerprinting.

The RAPD (Williams et al. 1990) amplification reactions were performed in a total volume of 25 µl containing 2.5 µl of 10X *Taq* buffer (Fermentas) (750 mM Tris-HCl, pH 8.8 at 25°C, 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20), 50 mM $MgCl_2$, 5 mM each of dATP, dCTP, dGTP and dTTP (SIGMA), 10 µl of random decamer primers and 1 unit of *Taq* DNA Polymerase (Fermentas). Four nanogram of template DNA was used for each amplification reaction in thermal cycler (BIO-RAD- My Cycler, Thermal Cycler). The first cycle was programmed as denaturation for 1 min at 94°C; annealing for 1 min at 37°C and extension for 1 min at 72°C. Subsequently 41 cycles were programmed as denaturation for 45 s at 94°C, annealing for 1 min at 37°C and extension for 1 min at 72°C. An additional extension at 72°C for 10 min was performed to facilitate complete extension.

Gel electrophoresis

Subsequently, amplification products were electrophoresed using 1.5% agarose gels with 1x TBE buffer at pH 8.0 for 3 h. Gel was visualized by 0.5 µg/ml ethidium bromide staining and photographed under UV light using Gel Documentation system (Gel-Doc-It System, UVP Ltd., Unit 1, Trinity Hall Estate, Nuffield Rd. Cambridge, CB4 1 TG, UK). Reagents and chemicals used in the present study were of analytical grades and obtained from Sigma (Sigma-Aldrich, Inc., 3050 Spruce street, St. Louis, MO, USA).

Data analysis

The RAPDs were scored as present (1) or absent (0) for the individual genotypes/clone. The data matrix obtained for presence or absence of RAPD bands was analyzed according to Nei and Li (1979) definition of genetic similarity, i.e. $S_{ij} = 2a/(2a+b+c)$ where S_{ij} is the similarity coefficient between two individuals (i and j), 'a' is the number of bands present in j and absent in i. In order to analyze the relatedness among the sources, the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) based dendrogram was constructed using Nei and Li genetic distance matrix (Nei and Li 1979) using NTSYSpc 2.2 software (Rohlf 1997). Genetic structure and measures of genetic diversity viz. total genetic diversity (H_t) and Shannon's Information index (I) was calculated as per the method of Nei (1973).

Polymorphic Information Content (PIC) was calculated using the formula PIC = 2p (1- p), where p is the frequency of the i^{th} allele (Roldan-Ruiz et al. 2000).

Marker index (MI) was calculated in order to characterize the capacity of each primer to detect polymorphic loci among the clones. MI was calculated as described by Varshney et al. (2007).

$$MI = PIC \times EMR$$

where, EMR (Effective Multiplex Ratio) is defined as the product of the fraction of polymorphic loci (β) and the number of polymorphic loci (n) for an individual assay.

$$EMR = n.\beta$$

Table 1. Detail of various clones of *D. sissoo* used for genetic analysis.

S. No.	Clone No.	Source / Origin	S. No.	Clone No.	Source / Origin
1	C1	Sabalgarh, Chiriyapur, Bijnor, U.P.	35	C168	Kanau, Chaur, Chila, Bairaj, U.P.
2	C2	Sabalgarh, Chiriyapur, Bijnor, U.P.	36	C169	Kanau, Chaur, Chila, Bairaj, U.P.
3	C3	Trilokpur, Gonda, Bijnor, U.P.	37	C174	Kanau, Chaur, Chila, Bairaj, U.P.
4	C4	Sabalgarh, Chiriyapur, Bijnor, U.P.	38	C175	Kanau, Chaur, Chila, Bairaj, U.P.
5	C9	Sabalgarh,Pathri, Haridwar, Uttarakhand	39	C192	Hasanpur 2, Tulsipur, Gonda, U.P.
6	C10	Sabalgarh,Pathri, Haridwar, Uttarakhand	40	C198	Hasanpur 2, Tulsipur, Gonda, U.P.
7	C11	Sabalgarh,Pathri, Haridwar, Uttarakhand	41	C201	Hasanpur 2, Tulsipur, North Gonda, U.P.
8	C12	Sabalgarh,Pathri, Haridwar, Uttarakhand	42	C204	Hasanpur 2, Tulsipur, Northgonda, U.P.
9	C14	Sabalgarh,Pathri, Haridwar, Uttarakhand	43	C232	Birpur 4A, Bhambar,Gonda, U.P.
10	C19	Shah Mansurpur, Sahranpur,U.P.	44	C235	Banketwa 2, Gonda, U.P.
11	C20	Shah Mansurpur, Sahranpur,U.P.	45	C237	Banketwa 2, Gonda, U.P.
12	C24	C.B.Ganj, Barielly,U.P	46	C361	Origin not known
13	C33	Bhainsaspur, Tulsipur, Gonda,U.P	47	W-1	Origin not known
14	C36	Hasanpur,Tulsipur, Gonda, U.P	48	W-2	Origin not known
15	C41	Hasanpur,Tulsipur, Gonda, U.P	49	S-2	Dhuri-Bagerian Road, Malerkotla, Sangrur, Punjab
16	C43	Trilokpur, Tulsipur, Gonda, U.P	50	S-7	Amargarh Chanuda Road, Malerkotla, Sangrur, Punjab
17	C46	Trilokpur, Tulsipur, Gonda, U.P	51	S-47	Patiala Sangrur Road, Sangrur, Punjab
18	C49	Trilokpur, Tulsipur, Gonda U.P.	52	S-65	Sangrur Barnala Road, Sangrur, Punjab
19	C51	Birpur, Bhambar,Gonda, U.P.	53	S-70	Bhatinda Branch Canal, Malerkotla, Sangrur, Punjab
20	C57	Dinsia, Khalawala, Ambala, Haryana	54	S-70A	BBC 70-71 L/S, Malerketla, Sangrur, Punjab
21	C60	Dinsia, Khalawala, Ambala, Haryana	55	S-70B	BBC 70-71 L/S, Malerketla, Sangrur, Punjab
22	C62	Chihrauli, Yamunanagar, Haryana	56	S-72	Bhatinda Branch Canal, Malerkotla, Sangrur, Punjab
23	C66	Chihrauli, Yamunanagar, Haryana	57	S-73	Bhatinda Branch Canal, Malerkotla, Sangrur, Punjab
24	C80	Hanumangarh, Sriganganagar, Rajasthan	58	S-179	Ghagar Branch Canal, Lahragaga, Sangrur, Punjab
25	C86	Narun Desal, Hanumangarh, Sriganganagar, Rajasthan	59	S-180	Ghagar Branch Canal, Lahragaga, Sangrur, Punjab
26	C88	Narun Desal, Hanumangarh, Sriganganagar, Rajasthan	60	S-194	Ghagar Branch Canal, Lahragaga, Sangrur, Punjab
27	C90	Lakhnwali, Hanumangarh, Sriganganagar, Rajasthan	61	S-199	Ghagar Branch Canal, Lahragaga, Sangrur, Punjab
28	C94	Lakhnwali, Hanumangarh, Sriganganagar, Rajasthan	62	S-DB	Dera Bassi, Patiala, Punjab
29	C103	RD, Suralgarh, Hanumangarh, Rajasthan	63	S-M1	Bir Mattawara, Ludhiana, Punjab
30	C107	Head Nursery Birdwal, Hanumangarh, Rajasthan	64	ABR-26	Abohar, Ferozepur, Punjab
31	C113	Hetunda Campus, Nepal	65	ABR-14	Abohar, Ferozepur, Punjab
32	C114	Hetunda Campus, Nepal	66	MG-21	Moga, Ferozepur, Punjab
33	C124	Kosi River Bank, Inerwa, Sarali, Nepal	67	MKT-161	Muktsar, Faridkot, Punjab
34	C128	Mahendragarh, Sarali, Nepal			

Results and discussion

The clones investigated under the study represent five Indian states viz. Haryana, Punjab, Rajasthan, Uttarakhand, Uttar Pradesh and neighboring country Nepal. The PCR amplification using 30 selected RAPD primers produced reproducible amplification products and was used to evaluate the level of genetic divergence between 67 clones of *D. sissoo*. The primers amplified different numbers of bands and demonstrated a comparable degree of polymorphism across the genotypes. RAPD analysis from different genotypes revealed a total of 342 bands, of which 290 were polymorphic, accounting for 78.8 % polymorphism. The number of bands per primer ranged from as low as 4 to a maximum 21, with an average of 11.4. The number of polymorphic bands varied from 1 (primer M-198) to 21 (primer OPA-08) (Table 2). Genetic divergence in respect of percent polymorphism ranged from 16.6% to 100% with an average of 78.8%. The primers OPA-17 & M-182 (Fig. 1a, b) showed highest polymorphism in various clones. The results revealed that the number of polymorphic primers and fragments generated was not of similar range and varied in different genotypes. This is understandable as product amplification depends upon the sequence of random primers and their compatibility with genomic DNA. The number of markers detected by each primer depends on primer sequence and the extent of genetic variation, which is genotype specific (Upadhyay et al. 2004).

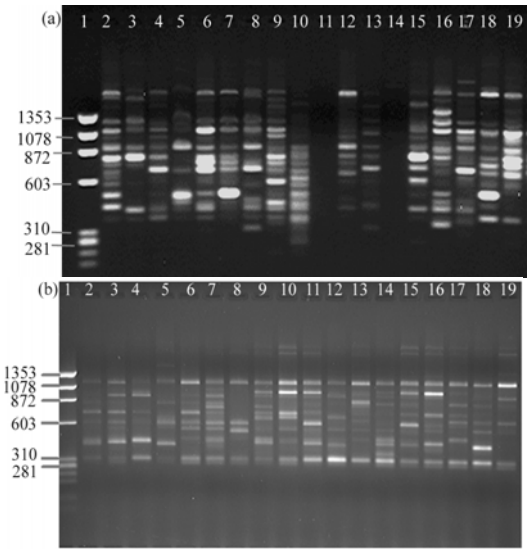

Fig. 1 (a) RAPD pattern obtained using primer OPA-17. Lane 1 is ϕX-174 DNA/BsuRI (HaeIII) molecular weight marker, lane 2 -19 comprised of C1, C2, C3, C4, C9, C10, C11, C12, C14, C19, C20, C24, C33, C36, C41, C43, C46, C49. (b)RAPD pattern obtained using primer M-182. Lane 1 is ϕX-174 DNA/BsuRI (HaeIII) molecular weight marker, lane 2 -19 comprised of C1, C2, C3, C4, C9, C10, C11, C12, C14, C19, C20, C24, C33, C36, C41, C43, C46, C49.

A number of marker attributes like PIC and MI have been used in several studies to assess the informative or discriminatory power of the primers for genetic diversity studies (Gupta and Varshney 2000; Varshney et al. 2007). The PIC value ranged from 0.01 (M-31) to 0.37 (OPA-17) with a mean of 0.17, while EMR ranged from 0.17 (M-198) to 21.00 (OPA-08) with a mean of 8.66 across all genotypes. The Marker Index (MI), which is considered as an overall measure of the efficiency to detect polymorphism (Varshney et al. 2007), ranged from 0.002 (M-31) to 7.42 (OPA-17). MI together with PIC value can be used to assess the informativeness of the primers. As shown in Table 2 primer M-31 was least polymorphic and thus informativeness of this primer is less as compared to other primers. Therefore marker index was also least for this primer. Maximum informativeness could be obtained for the primer OPA-17, also the marker index was highest for it. Effective multiplex ratio (EMR), is the number of polymorphic loci per primer (Ude et al. 2003). Effective multiplex ratio (EMR) was found maximum for primer OPA-08 and minimum for primer M-198.

The dendrogram based on UPGMA analysis grouped 67 clones of shisham into six clusters of which three were major (cluster II, III & IV) and three small (minor) clusters (Cluster I, V, VI) (Fig. 2). The first cluster (cluster I) consisted of three clones viz. C1, C2 and C33 all from nearby areas from the state of Uttar Pradesh (U.P.). The cluster II comprises of 14 clones viz. C3, C43, C46, C49, C51, C36, C41, C9, C11, C10, C20, C12, C4 and C24 predominantly from U.P. and Uttarakhand states. The highest similarity in this cluster was found between clone C49 and C51 depicting a similarity index of 0.912 (Appendix 1). If we look at the geographical occurrence of these clones, they mostly originate from the administrative area of one state i.e. U.P. It is expected also that the clustering grouped all closely related clones together in one cluster. The Cluster III consists of 18 clones predominantly from the states of U.P. and Punjab. This cluster is divided into two sub-clusters, sub-cluster 1 and sub-cluster 2. The highest similarity in this cluster was found between clone S70B and S72 depicting a similarity index of 0.888 (Appendix 1). Sub cluster 1 comprises 13 clones viz. C192, C201, S-70B, S-72, C232, C237, C204, C235, S-7, S-47, S-70, S-65 and S-70A, while Sub cluster 2 consists of genotypes S-180, S-199, SM-1, and MG-21. Cluster IV consists of 16 clones predominantly from the states of Rajasthan, Haryana and U.P. The clones of Nepal also represented this cluster. The highest similarity in this cluster was found between clone C94 and C103 depicting a similarity index of 0.896 (Appendix 1). This cluster is divided into two sub-clusters, sub-cluster 3 & 4. Sub cluster 3 comprises of three clones C57, C107, and C113 from Haryana, Rajasthan and Nepal, respectively. Sub cluster 4 consists of 13 clones C60, C90, C128, C62, C94, C103, C114, C124, C174, C175, C80, C88 and C168. Cluster V (small cluster) consisted of three clones namely C169, C361, and W-1. The sixth cluster consists of five clones viz. S-179, S-194, ABR-26, ABR-14 and MKT-161, all representing from one state i.e. Punjab.

In the entire dendrogram there were many closely related clones having high similarity and low genetic distance. Some of the closely related clones are C49 and C51, C36 and C41, C9 and C11, C192 and C201, S70B and S72, S47 and S70, S180 and S199, C60 and C90, C94 and C103, etc. with similarity index of 0.912, 0.901, 0.885, 0.888, 0.888, 0.882, 0.880, 0.888, 0.896, respectively (Appendix 1). Other genetically similar clones found were C198 and W-2, C19 and S-2, C66 and C86, with similarity index of 0.759, 0.784 and 0.775, respectively, though they did not group in any major, minor or sub-cluster. The most closely related clones were C49 and C51 with similarity index of 0.912 while the least similar or most dissimilar genotypes were C14 and S-DB showing similarity index of 0.685 (Appendix 1). As per the geographical locations of those clones, these are closely located and it is assumed that there is more homogeneity/similarity between them. It is recommended that while selecting clones for deployment, the most divergent clones should be used. This will help to maintain diversity in the plantations.

The genetic diversity varied from 0.012 to 0.37 with an average of 0.19. Shannon's Information index (I) varied between 0.026 (M-31) and 0.544 (OPA-17). The average value of the index was 0.310 (Table 2). Shannon's Information index (I) is a diversity index or a mathematical measure of species diversity in a community. Diversity indices provide important information about rarity and commonness of alleles in a species or genotype. On comparison with other tropical forest tree species, the gene diversity in *D. sissoo* was comparatively low (Table 3). The range of genetic diversity is far lower than the gene diversity in other Indian tropical tree species like Neem (0.2–0.88) (Deshwal et al. 2005), *Butea monosperma* (0.57–0.79) (Vaishali et al. 2008), *Prosopis juliflora* (0.18–0.75) (Vaishali et al. 2008), Date Palm (0.02–0.77) (Zehdi et al. 2004), Acacia (0.09–0.31) (Nanda

et al. 2004).

Table 2. RAPD polymorphism and genetic diversity in *D. sissoo* clones.

S. No.	Primer	Total number of bands	Number of polymorphic bands	Percent polymorphic bands	Total gene diversity (Ht)	Shannon's Information index (I)	Polymorphic Information Content (PIC)	Effective Multiplex Ratio (EMR)	Marker index (MI)
1	M-29 (CCGGCCTTAC)	14	13	92.86	0.2346	0.3547	0.23	12.07	2.83
2	M-31 (CCGGCCTTCC)	5	1	20.00	0.0116	0.0268	0.01	0.20	0.002
3	M-33 (CCGGCTGGAA)	19	18	94.74	0.2372	0.3703	0.24	17.05	4.05
4	M-119 (ATTGGGCGAT)	6	4	66.67	0.0373	0.0773	0.04	3.57	0.13
5	M-122 (GTAGACGAGC)	10	5	50.00	0.0211	0.0500	0.02	2.50	0.05
6	M-132 (AGGGATCTCC)	6	2	33.33	0.0275	0.0542	0.03	0.67	0.02
7	M-147 (GTGCGTCCTC)	15	11	73.33	0.2217	0.3346	0.22	8.07	1.79
8	M-156 (GCCTGGTTGC)	10	6	60.00	0.1626	0.2538	0.16	3.60	0.59
9	M-182 (GTTCTCGTGT)	15	15	100.00	0.3144	0.4716	0.31	13.07	4.11
10	M-186 (GTGCGTCGCT)	4	4	100.00	0.0432	0.0976	0.04	4.00	0.17
11	M-188 (GCTGGACATC)	7	4	57.14	0.1218	0.1921	0.12	2.29	0.28
12	M-198 (GCAGGACTGC)	6	1	16.67	0.0551	0.0854	0.06	0.17	0.01
13	M199 (GCTCCCCCAC)	5	2	40.00	0.0283	0.0607	0.03	0.80	0.02
14	OPA-02 (TGCCGAGCTG)	17	16	94.12	0.2834	0.4318	0.28	15.06	4.27
15	OPA-04 (AATCGGGCTG)	12	11	91.67	0.2838	0.4314	0.28	10.08	2.86
16	OPA-05 (AGGGGTCTTG)	9	8	88.89	0.1047	0.1851	0.10	7.11	0.74
17	OPA-06 (GGTCCCTGAC)	11	10	90.91	0.2597	0.3958	0.26	9.09	2.36
18	OPA-07 (GAAACGGGTG)	11	7	63.64	0.0902	0.1578	0.09	4.45	0.40
19	OPA-08 (GTGACGTAGG)	21	21	100.00	0.3246	0.4935	0.32	21.00	6.82
20	OPA-09 (GGGTAACGCC)	15	14	93.33	0.2373	0.3702	0.24	13.07	3.10
21	OPA-10 (GTGATCGCAG)	12	11	91.67	0.2423	0.3662	0.24	10.08	2.44
22	OPA-11 (CAATCGCCGT)	11	7	63.64	0.0923	0.1587	0.09	4.45	0.41
23	OPA-14 (TCTGTGCTGG)	12	10	83.33	0.1769	0.2785	0.18	8.33	1.47
24	OPA-16 (AGCCAGCGAA)	10	10	100.00	0.3502	0.5259	0.35	10.00	3.50
25	OPA-17 (GACCGCTTGT)	20	20	100.00	0.3708	0.5440	0.37	20.00	7.42
26	OPA-18 (AGGTGACCGT)	17	17	100.00	0.1349	0.2521	0.13	17.00	2.29
27	OPA-20 (GTTGCGATCC)	10	10	100.00	0.2793	0.4426	0.28	10.00	2.79
28	OPAF-07 (GGAAAGCGTC)	11	11	100.00	0.1016	0.1888	0.10	11.00	1.12
29	OPAF-16 (TCCCGGTGAG)	12	12	100.00	0.2393	0.3683	0.24	12.00	2.87
30	OPG-09 (CTGACGTCAC)	9	9	100.00	0.1625	0.2811	0.16	9.00	1.46
	Total	342.00	290.00	-	5.2499	8.3012	5.25	259.78	60.38
	Min	4.00	1.00	16.67	0.0116	0.0268	0.01	0.17	0.002
	Max	21.00	21.00	100.00	0.3708	0.5440	0.37	21.00	7.42
	Average	11.40	9.67	78.86	0.1986 (0.0335)	0.3106 (0.2534)	0.17	8.66	2.01

Table 3. Comparison of genetic diversity of *D. sissoo* with other tropical tree speices using dominant marker sets.

Species	Geographic distribution	Putative mating system	Genetic diversity estimate (Mean)	Number of populations	Number of trees / accessions
*Dalbergia sissoo (*Present study*)*	Northern India	Mixed mating	0.19	-	67
Anacardium occidentale (Kremer et al., 2005)	Amazonian basin, northeast and central Brazil	Out crossing	0.32	2	89
Butea monosperma (Vaishali et al., 2008)	India	-	0.43	-	16
Cedrela odorata (Cavers et al, 2004)	Mexico to south Brazil	Out crossing	0.30	9	125
Chrisophyllum sanguinolentum (Caron et al., 2004)	Amazonia	Out crossing	0.17	1	51
Eperua grandiflora (Caron et al., 2004)	Amazonia	Out crossing	0.17	1	47
Eucalyptus grandis (Okun et al., 2008)	Keniya	Out crossing	0.26	6	-
Eugenia uniflora (Margis et al., 2002)	Atlantic coast of Brazil to Paraguay and Argentina	Out crossing	0.23	5	78
Pseudobombax munguba (Kremer et al., 2005)	Amazonia	Mixed mating	0.14	9	168
Swietenia macrophylla (Lowe et al., 2003)	Mexico to south Brazil	Out crossing	0.13	3	110
Virola michelii (Caron et al., 2004)	Guiana shield	Out crossing	0.31	1	37
Voschysia ferruginea (Kremer et al., 2005)	Mexico to south Brazil	Mixed mating	0.31	9	262

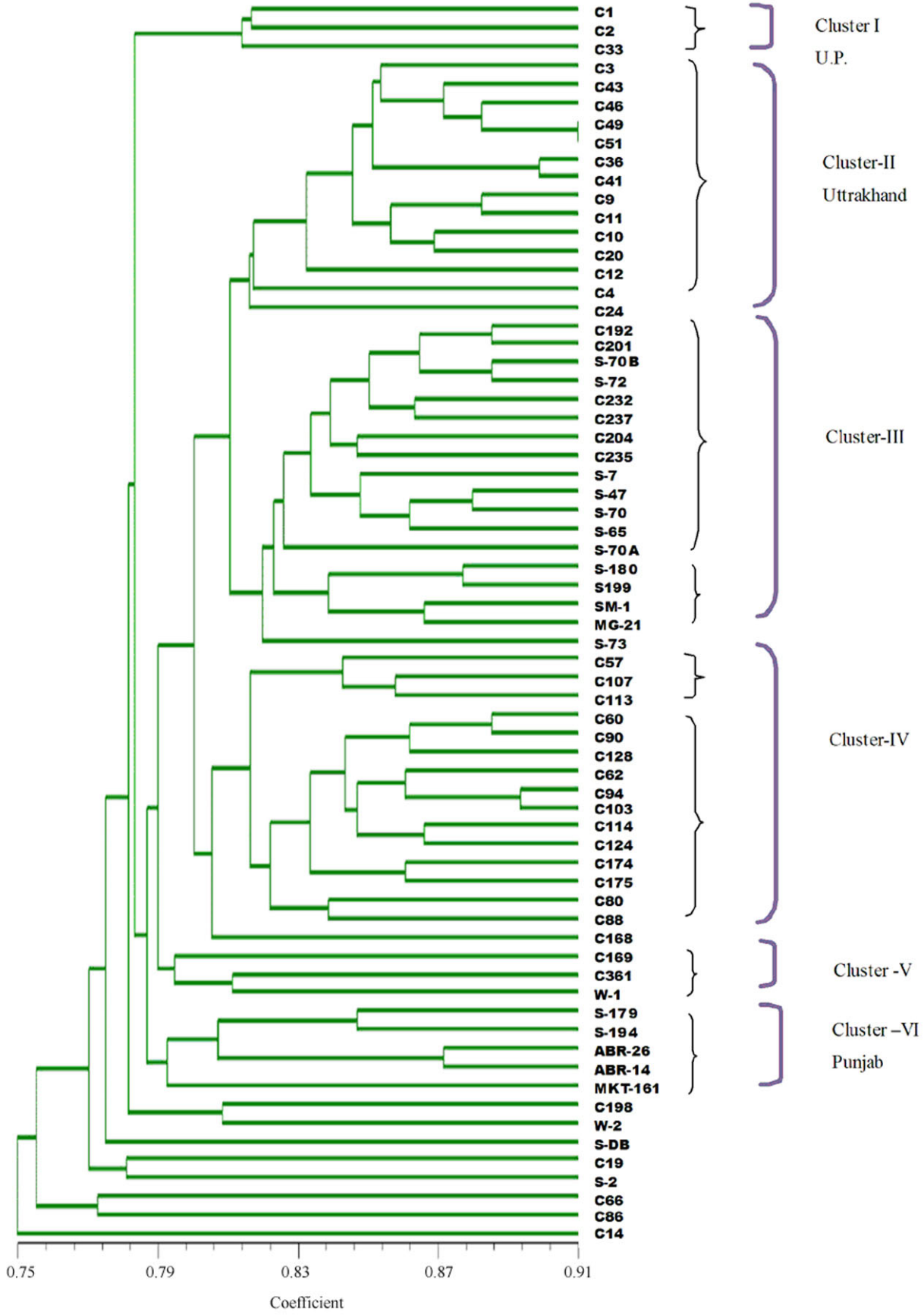

Fig. 2 Genetic relationship between different clones of *D.sissoo* based on UPGMA cluster analysis

Variation in genetic diversity is usually related with geographical range, mode of reproduction, mating system, seed dispersal and fecundity. Ashraf et al. (2010) studied genetic diversity of 22 accessions of *D. sissoo* collected from the canal road, farmer's field and forest sites of N.W.F.P, Pakistan using RAPD markers. Their results also indicate that the mean level of genetic variation was low among the individuals of *D. sissoo.* Rout et al. (2003) also obtained similar results in *Dalbergia* species using RAPD markers. Since shisham forests have been subjected to selective felling and other type of deforestation, remnant populations naturally distributed in public and farm areas are more seriously exposed to human activities than those in forest reserves. The populations within public and farm areas are generally smaller in size than those in natural forests. Under the situation limited numbers of trees are involved in mating. This might cause inbreeding depression, which could reduce variation within populations.

The low level of genetic diversity suggests that regional strategies are required for conservation of *D. sissoo* germplasm in the country in general and maintaining wide basket of diversity in plantations in particular for addressing the problems related the mortality of shisham in the country. This result indicates the potential use of these, particularly the more distant genotypes (clones), for future breeding and plantation programs. Because significant intra-specific variation has been demonstrated in this species (Shiv Kumar and Banerjee 1986; Dhillon et al. 1995; Sagta and Nautiyal 2001) and better adaptable and productive provenances have been identified, hence the selected provenances will be of the interest to establish several plantations of 10 to 50 ha of each tested provenances. Those plantations would be converted later to seed production area by early and heavy thinning as suggested by Boland (1981) in case of narrow genetic base species like Eucalyptus. This would serve as ex-situ conservation stands, produce large quantities of seed and provide reasonably broad genetic base for several generations. There is also a need to maintain sufficiently large populations in natural habitats to conserve genetic diversity in *D. sissoo* and avoid genetic erosion through encroachment and illicit felling.

The wise use of clones can ensure genetic diversity in the plantations and it can be more effectively maintained in clonal forestry than in traditional approaches, by deliberately selecting highly productive but divergent and unrelated clones, rather than the use of related seedlings from a seed stand/seed orchard (Heybroek 1978; Libby 1985). The best strategy is to plant several genetically distant and unrelated clones with only relatively few individuals (ramets) per clone (Liby 1982). Though a mixture of large numbers of clones is a safe option but the genetic gains will not be as high as could be achieved by fewer clones. Hence a mixture of about 20–30 genetically tested and unrelated clones are probably the optimal strategy to maintain the genetic diversity in plantations. The most divergent clones as evident from the investigation could be taken up for clonal forestry program and for the development of new recombinants through intra-species hybridization. Deployment and maintenance of genetically divergent and unrelated clones will be an important step to combat shisham mortality in plantations, and in particular planting sets of 20–30 genetically dissimilar clones in mixture will maintain a wider genetic base in *D. sissoo*.

Acknowledgements

The authors are thankful to Punjab Forest Department for providing financial support for conducting this study.

References

Arif M, Zaidi NW, Singh YP, Haq QMR, Singh US. 2009. A comparative analysis of ISSR and RAPD markers for study of genetic diversity in Shisham (*Dalbergia sissoo*). *Plant Mol Biol Rep*, **27**(4): 488–495.

Ashraf M, Abdul SM, Rabia R, Sobia T. 2010. A molecular study of genetic diversity in shisham (*dalbergia sissoo*) plantation of NWFP, Pakistan. *Pakistan Journal of Botany*, **42**(1): 79–88.

Boland DJ. 1981. Eucalypt seed for Indian populations from better Australian natural seed sources. *Indian Forester*, **107**(3): 125−134.

Caron H, Bandou E, Kremer A. 2004. Multilocus assessment of levels of genetic diversity in tropical trees in Paracou stands. In: Gourlet FS, Guehl JM, Laroussinie O (eds), *Ecology and Management of a Neotropical Rainforest*. Amsterdam: Elsevier, Pp. 160−171.

Castiglione S, Wang G, Damini G, Bandi C, Bisoffi S, Sala F. 1993. RAPD fingerprints for identification and for taxonomic studies of elite poplar (*Populus* spp.) clones. *Theor Appl Genet*, **87**: 54−59.

Cavers S, Navarro C, Lowe AJ. 2004. A combination of molecular markers (cpDNA, PCR-RFLP, AFLP) identifies evolutionarily significant units in *Cedrela odorata* L. (Meliaceae) in Costa Rica. *Conserv Genet*, **4**: 571−580.

Deshwal RPS, Singh R, Malik K, Randhawa GJ. 2005. Assessment of genetic diversity and genetic relationships among 29 population of *Azadirachta indica* A. Jess. using RAPD markers. *Genetic Resources and Crop Evolution*, **52**: 285−292.

Dhillon RS, Bisla, Dhanda SK, Singh VP. 1995. Genetic variation and heritability of some growth traits in shisham (*Dalbergia sissoo* ROXB.). *Annals of Biol*, **11**(1): 107–110.

Esselman EJ, Li JQ, Crawford D, Winduss JL, Wolfe AD. 1999. Clonal diversity in the rare *Calamagrostis porteri* ssp. Insperata (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers. *Mol Ecol*, **8**: 443–451.

Ginwal HS, Maurya SS. 2008. RAPD markers for genetic diversity in *Eucalyptus tereticornis* Sm. of Australian and Papua New Guinea origin. In: Ansari SA, Narayanan C, Mandal AK (eds.), *Forest Biotechnology in India*. Delhi: Satish Serial Publishing House, 75−87.

Ginwal HS, Maurya SS. 2010. Evaluation and optimization of DNA extraction method for *Dalbergia sissoo* leaf. *Indian Journal of Biotechnology*, **9**: 69−73.

Gupta PK, Varshney RK. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica*, **113**: 163–185.

Heybroek HM. 1978. Primary considerations: multiplication and genetic diversity. *Unasylva* **30**: 27−33.

Keil M, Griffin AR. 1994. Use of random amplified polymorphic DNA (RAPD) markers in the discrimination and verification of genotypes in Eucalyptus. *Theor Appl Genet*, **88**: 116−122.

Kremer A, Caron H, Cavers S, Colpaert N, Gheysen G, Gribel R, Lemes M, Lowe AJ, Margis R, Navarro C, Salgueiro F. 2005. Monitoring genetic

diversity in tropical trees with multilocus dominant markers. *Heredity*, **95**: 274–280.

Li A, Ge S. 2001. Genetic variation and clonal diversity of *Psammochloa villosa* (Poaceae) detected by ISSR markers. *Ann Bot*, **87**: 585−590.

Libby WJ. 1982. What is a safe number of clones per plantation? In: H.M. Heybroek, B.R. Stephan and K. von Weissenberg (Eds.), *Resistance to Diseases and Pests.* The Netherlands: Pudoc. Wageningen, pp. 342−360.

Libby WJ. 1985. Potential of clonal forestry. In: L. Zsuffa, R.M. Rauter, and C.W. Yeatman (Eds.), *Clonal Forestry: its Impact on Tree Improvement and our Future Forests.* Proc. 19th Meeting Canadian Tree Improvement Association, Part 2, pp.1-11.

Lowe AJ, Jourde B, Breyne P, Colpaert N, Navarro C, Cavers S. 2003. Fine scale genetic structure and gene flow within Costa Rican populations of Mahogany (*Swietenia macrophylla*). *Heredity*, **90**: 268−275.

Margis R, Felix DB, Caldas JF, Salgueiro F, Dearaujo DSD, Breyne P, et al. 2002. Biodiversity on three neighboring populations of *Eugenia uniflora* (pitanga) from Brazilian Atlantic rain forest accessed by AFLP markers. *Biodivers and Conserv*, **11**: 149−163.

Mosseler A, Egger KN, Hughes GA. 1992. Low levels of genetic diversity in red pine confirmed by Random Amplified Polymorphic DNA markers. *Can J For Res*, **22**: 1332−1337.

Nanda MR, Nayak S, Rout GR. 2004. Studies on genetic relatedness of *Acacia* tree species using RAPD markers. *Biologia Bratislava*, **59**: 115−120.

Nei M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences, USA **70**: 3321–3323.

Nei M, Li WH. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Nat Acad Sci USA*, **76**: 5269−5273.

Okun DO, Kenya EU, Oballa PO, Odee DW, Muluvi GM. 2008. Analysis of genetic diversity in *Eucalyptus grandis* (*Hill* ex Maiden) seed sources using inter simple sequence repeats (ISSR) molecular markers. *African Journal of Biotechnology*, **7**(13): 2119−2123.

Parajuli AV, Bhatta B, Adhikari MK, Tuladhar J, Thapa HB. 1999. Causal agents responsible for the die-back of *Dalbergia sissoo* in Nepal's eastern Terai. *Ban Ko Janakari*, **9**(1).

Parsons BJ, Newbury HJ, Jackson MT, Ford-Lloyd BV. 1997. Contrasting genetic diversity relationships are revealed in rice (*Oryza sativa* L.) using different marker types. *Mol Breed*, **3**: 115–125.

Rajgopal J, Bashyam L, Bhatia S, Khurana DK, Srivastava PS, Lakshmikumaran M. 2000. Evaluation of genetic diversity in the Himalyan Poplar using RAPD markers. *Silvae Genetica*, **49**(2): 60−66.

Rohlf FJ. 1997. NTSYSpc: numerical taxonomy and multivariate analysis system, version 2.02. Exeter Software, Setauket, New York, USA.

Roldan-Ruiz I, Dendauw J, Vanbockstaele E, Depicker A, De loose M. 2000. AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). *Mol Breed*, **6**: 125−134.

Rout GR, Bhatacharya D, Nanda RM, Nayak S, Das P. 2003. Evaluation of genetic relationships in *Dalbergia* species using RAPD markers. *Biodiversity and Conservation*, **12**: 197−206.

Sagta HC, Nautiyal. 2001. Growth performance and genetic divergence of various provenances of *Dalbergia Sissoo* ROXB. at nursery stage. *Silvae Genetica*, **50**(3-4): 93−99.

Scheepers D, Eloy MC, Briquet M. 1997. Use of RAPD patterns for clone verification and in studying provenance relationship in Norway spruce (*Picea abies*). *Theor Appl Genet*, **94**: 480−485.

Schnell RJ, Ronning CM, Knight RJ,Jr . 1995. Identification of cultivars and validation of genetic relationship in *Mangifera indica* L. using RAPD markers. *Theor Appl Genet*, **90**: 269−274.

Shiv-Kumar P, Banerjee AC. 1986. Provenance trials of *Acacia nilotica*. *J Tree Sci*, **5**(1): 53−56.

Ude GN, Kenworthy WJ, Costa JM, Cregan PB, Alvernaz J. 2003. Genetic diversity of soybean cultivars from China, Japan, North America, and North American ancestral lines determined by amplified fragment length polymorphism. *Crop Sci*, **43**: 1858−1867.

Upadhyay A, Jayadev K, Manimekalai R, Parthasarathy VA. 2004. Genetic relationship and diversity in Indian coconut accessions based on RAPD markers. *Scientia Horticulturae*, **99**: 353–362.

Vaishali, Khan S, Sharma V. 2008. RAPD based assessment of genetic diversity of *Butea monosperma* from different agro-ecological regions of India. *Indian Journal of Biotechnology*, **7**: 320−327.

Varshney RK, Chabane K, Hendre PS, Aggarwal RK, Graner A. 2007. Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barleys. *Plant Sci,***173**: 638–649.

Whitkus R, Cruz ML, Bravo M, Gomez-Pompa A. 1998. Genetic diversity and relationships of cacao (*Theobroma cacao* L.) in southern Mexico. *Theor Appl Genet*, **96**: 621−627.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nuc Ac Res*, **18**: 6531−6535.

Zehdi S, Sakka H, Mohamed Salem AO, Rhouma A, Marrakchi M, Trifi M. 2004. Molecular polymorphism and genetic relationships in a Tunisian date palm (*Phoenix dactylifera* L.) collection using ISSR amplification fingerprinting. *IPGR News Lett*, **144**: 39−44.

Appendix 1: Genetic similarity index between various clones of *D. sissoo.*

	C1	C2	C3	C4	C9	C10	C11	C12
C1	1							
C2	0.819	1						
C3	0.852	0.83	1					
C4	0.811	0.811	0.833	1				
C9	0.836	0.814	0.863	0.838	1			
c10	0.789	0.756	0.827	0.808	0.86	1		
c11	0.814	0.797	0.836	0.827	0.885	0.849	1	
c12	0.819	0.775	0.841	0.778	0.858	0.806	0.852	1
c14	0.748	0.781	0.797	0.756	0.786	0.751	0.759	0.781
c19	0.74	0.756	0.762	0.77	0.773	0.764	0.762	0.756
c20	0.814	0.781	0.858	0.838	0.885	0.871	0.841	0.819
c24	0.767	0.778	0.833	0.803	0.816	0.792	0.822	0.8
c33	0.816	0.816	0.811	0.797	0.806	0.792	0.8	0.8
c36	0.8	0.756	0.849	0.814	0.833	0.819	0.838	0.855
c41	0.822	0.795	0.849	0.819	0.855	0.847	0.844	0.849
c43	0.833	0.778	0.849	0.819	0.838	0.847	0.849	0.849
c46	0.816	0.795	0.844	0.83	0.871	0.852	0.827	0.822
c49	0.825	0.797	0.863	0.816	0.858	0.844	0.841	0.814
c51	0.819	0.803	0.869	0.816	0.88	0.838	0.841	0.819
c57	0.803	0.781	0.797	0.8	0.797	0.762	0.808	0.781
c60	0.814	0.737	0.814	0.806	0.83	0.816	0.83	0.786
c62	0.806	0.806	0.833	0.819	0.822	0.803	0.844	0.811
c66	0.723	0.745	0.762	0.737	0.756	0.732	0.751	0.729
c80	0.811	0.8	0.838	0.792	0.833	0.808	0.838	0.816
c86	0.734	0.718	0.762	0.748	0.756	0.748	0.762	0.773
c88	0.784	0.767	0.8	0.781	0.795	0.797	0.806	0.778
c90	0.789	0.745	0.811	0.781	0.833	0.803	0.811	0.8
c94	0.808	0.764	0.825	0.8	0.836	0.811	0.83	0.803
c103	0.819	0.77	0.814	0.784	0.83	0.789	0.819	0.819
c107	0.784	0.773	0.789	0.781	0.795	0.775	0.816	0.8
c113	0.797	0.748	0.786	0.734	0.775	0.734	0.781	0.803
c114	0.808	0.753	0.803	0.762	0.808	0.773	0.786	0.836
c124	0.786	0.764	0.825	0.767	0.836	0.827	0.803	0.847
c128	0.786	0.732	0.803	0.767	0.852	0.816	0.819	0.819
c168	0.756	0.74	0.795	0.748	0.784	0.764	0.789	0.789
c169	0.792	0.803	0.803	0.811	0.814	0.767	0.792	0.786
c174	0.786	0.753	0.803	0.8	0.819	0.822	0.797	0.792
c175	0.819	0.775	0.797	0.778	0.825	0.784	0.797	0.83
c192	0.8	0.773	0.838	0.786	0.86	0.869	0.833	0.838
c198	0.759	0.748	0.797	0.762	0.819	0.8	0.814	0.781
c201	0.797	0.77	0.814	0.806	0.847	0.833	0.814	0.808
c204	0.781	0.748	0.803	0.784	0.786	0.762	0.797	0.814
c232	0.808	0.77	0.803	0.789	0.836	0.767	0.792	0.825
c235	0.773	0.773	0.795	0.781	0.816	0.77	0.806	0.784
c237	0.822	0.778	0.827	0.792	0.86	0.825	0.816	0.816
c361	0.786	0.737	0.792	0.778	0.808	0.789	0.808	0.786
W-1	0.775	0.759	0.786	0.795	0.797	0.778	0.797	0.753
W-2	0.795	0.773	0.773	0.77	0.751	0.743	0.784	0.762
S-2	0.77	0.753	0.759	0.773	0.808	0.795	0.781	0.775
S-7	0.789	0.773	0.811	0.803	0.844	0.819	0.833	0.811
S-47	0.797	0.792	0.797	0.811	0.819	0.784	0.825	0.808
S-65	0.808	0.797	0.792	0.822	0.825	0.784	0.803	0.825
S-70	0.773	0.789	0.811	0.814	0.822	0.814	0.806	0.778
S-70A	0.773	0.762	0.789	0.814	0.816	0.775	0.811	0.767

	C1	C2	C3	C4	C9	C10	C11	C12
S-70B	0.8	0.789	0.822	0.819	0.86	0.808	0.816	0.811
S-72	0.83	0.808	0.836	0.822	0.836	0.795	0.808	0.819
S-73	0.781	0.759	0.803	0.773	0.847	0.795	0.825	0.814
S-179	0.786	0.77	0.803	0.789	0.819	0.762	0.792	0.764
S-180	0.786	0.786	0.814	0.806	0.836	0.816	0.836	0.786
S-194	0.756	0.756	0.773	0.775	0.773	0.748	0.767	0.784
S-199	0.775	0.77	0.797	0.778	0.83	0.773	0.814	0.781
S-DB	0.773	0.74	0.767	0.753	0.795	0.748	0.773	0.778
S-M1	0.778	0.767	0.789	0.764	0.811	0.77	0.806	0.8
ABR-26	0.762	0.773	0.784	0.77	0.795	0.743	0.789	0.773
ABR-14	0.778	0.767	0.811	0.775	0.822	0.781	0.8	0.811
MG-21	0.811	0.811	0.822	0.808	0.838	0.775	0.811	0.822
MKT-161	0.756	0.762	0.773	0.781	0.806	0.759	0.767	0.767

	C14	C19	C20	C24	C33	C36	C41	C43
c14								
c19	0.712	1						
c20	0.786	0.811	1					
c24	0.811	0.737	0.822	1				
c33	0.795	0.77	0.811	0.792	1			
c36	0.8	0.759	0.838	0.825	0.83	1		
c41	0.778	0.781	0.86	0.819	0.825	0.901	1	
c43	0.778	0.775	0.849	0.83	0.83	0.863	0.869	1
c46	0.773	0.792	0.866	0.814	0.83	0.858	0.874	0.874
c49	0.786	0.795	0.869	0.833	0.816	0.844	0.849	0.888
c51	0.77	0.8	0.858	0.833	0.806	0.833	0.849	0.86
c57	0.721	0.773	0.808	0.751	0.767	0.773	0.789	0.8
c60	0.726	0.778	0.819	0.773	0.778	0.822	0.827	0.833
c62	0.74	0.775	0.827	0.792	0.786	0.83	0.814	0.814
c66	0.751	0.699	0.745	0.781	0.726	0.759	0.759	0.748
c80	0.751	0.808	0.822	0.786	0.781	0.819	0.819	0.814
c86	0.707	0.732	0.745	0.748	0.715	0.792	0.786	0.792
c88	0.718	0.775	0.806	0.753	0.748	0.781	0.825	0.808
c90	0.734	0.77	0.811	0.786	0.77	0.836	0.836	0.819
c94	0.726	0.795	0.83	0.745	0.756	0.816	0.838	0.827
c103	0.753	0.756	0.797	0.773	0.762	0.8	0.827	0.822
c107	0.756	0.775	0.767	0.764	0.759	0.775	0.781	0.792
c113	0.732	0.745	0.759	0.756	0.74	0.789	0.773	0.8
c114	0.764	0.751	0.786	0.756	0.773	0.795	0.822	0.811
c124	0.743	0.806	0.825	0.778	0.756	0.816	0.838	0.833
c128	0.748	0.778	0.83	0.778	0.756	0.806	0.822	0.816
c168	0.734	0.737	0.767	0.781	0.748	0.803	0.814	0.836
c169	0.77	0.773	0.775	0.762	0.795	0.784	0.811	0.811
c174	0.737	0.795	0.819	0.767	0.8	0.833	0.838	0.833
c175	0.748	0.773	0.814	0.778	0.8	0.822	0.833	0.827
c192	0.745	0.77	0.86	0.775	0.803	0.825	0.863	0.841
c198	0.753	0.756	0.786	0.795	0.751	0.784	0.784	0.8
c201	0.759	0.795	0.869	0.773	0.806	0.816	0.833	0.816
c204	0.753	0.745	0.797	0.773	0.795	0.8	0.8	0.811
c232	0.759	0.773	0.814	0.767	0.773	0.795	0.806	0.789
c235	0.751	0.748	0.795	0.775	0.786	0.781	0.814	0.808
c237	0.74	0.775	0.838	0.781	0.814	0.808	0.836	0.819
c361	0.715	0.734	0.786	0.751	0.762	0.789	0.806	0.795
W-1	0.737	0.74	0.797	0.784	0.756	0.795	0.811	0.811
W-2	0.74	0.743	0.762	0.781	0.775	0.77	0.77	0.786

Continued the Appendix

	C14	C19	C20	C24	C33	C36	C41	C43
S-2	0.759	0.784	0.803	0.745	0.784	0.773	0.789	0.778
S-7	0.756	0.781	0.844	0.814	0.797	0.808	0.819	0.825
S-47	0.721	0.789	0.83	0.767	0.784	0.8	0.816	0.827
S-65	0.764	0.784	0.836	0.784	0.811	0.8	0.816	0.816
S-70	0.745	0.77	0.855	0.803	0.759	0.792	0.808	0.819
S-70A	0.745	0.748	0.806	0.792	0.775	0.797	0.819	0.808
S-70B	0.778	0.797	0.838	0.803	0.792	0.83	0.852	0.825
S-72	0.759	0.773	0.83	0.784	0.811	0.833	0.86	0.844
S-73	0.748	0.767	0.825	0.784	0.778	0.795	0.838	0.8
S-179	0.748	0.767	0.836	0.756	0.773	0.773	0.789	0.784
S-180	0.759	0.767	0.836	0.795	0.795	0.795	0.833	0.833
S-194	0.756	0.77	0.795	0.743	0.753	0.775	0.792	0.786
S-199	0.753	0.734	0.792	0.795	0.778	0.789	0.795	0.816
S-DB	0.685	0.726	0.773	0.743	0.737	0.764	0.764	0.781
S-M1	0.707	0.748	0.784	0.759	0.759	0.792	0.803	0.803
ABR-26	0.8	0.732	0.789	0.748	0.77	0.753	0.77	0.764
ABR-14	0.751	0.753	0.811	0.764	0.781	0.786	0.803	0.797
MG-21	0.751	0.786	0.795	0.797	0.781	0.792	0.825	0.792
MKT-161	0.723	0.753	0.795	0.764	0.748	0.781	0.786	0.786

	C46	C49	C51	C57	C60	C62	C66	C80
c46	1							
c49	0.877	1						
c51	0.893	0.912	1					
c57	0.784	0.814	0.797	1				
c60	0.827	0.819	0.825	0.863	1			
c62	0.814	0.822	0.827	0.844	0.849	1		
c66	0.77	0.767	0.778	0.762	0.795	0.792	1	
c80	0.836	0.822	0.838	0.816	0.855	0.858	0.748	1
c86	0.775	0.8	0.784	0.745	0.795	0.803	0.775	0.77
c88	0.786	0.806	0.795	0.816	0.838	0.825	0.743	0.841
c90	0.814	0.827	0.827	0.806	0.888	0.836	0.792	0.836
c94	0.827	0.841	0.825	0.83	0.847	0.86	0.756	0.855
c103	0.822	0.825	0.819	0.83	0.847	0.866	0.773	0.822
c107	0.797	0.795	0.795	0.833	0.822	0.841	0.77	0.852
c113	0.756	0.814	0.786	0.858	0.797	0.806	0.751	0.816
c114	0.8	0.803	0.797	0.808	0.836	0.833	0.784	0.806
c124	0.811	0.836	0.852	0.797	0.852	0.838	0.767	0.827
c128	0.811	0.841	0.841	0.819	0.869	0.822	0.773	0.822
c168	0.819	0.827	0.816	0.778	0.806	0.792	0.759	0.808
c169	0.784	0.797	0.797	0.814	0.808	0.8	0.751	0.789
c174	0.849	0.836	0.852	0.814	0.874	0.844	0.806	0.811
c175	0.822	0.814	0.814	0.808	0.814	0.827	0.789	0.822
c192	0.836	0.844	0.838	0.816	0.838	0.825	0.759	0.814
c198	0.816	0.83	0.803	0.764	0.781	0.795	0.795	0.784
c201	0.855	0.814	0.819	0.808	0.847	0.827	0.767	0.8
c204	0.795	0.797	0.77	0.77	0.781	0.8	0.756	0.751
c232	0.827	0.803	0.797	0.764	0.775	0.784	0.756	0.8
c235	0.814	0.806	0.8	0.762	0.784	0.786	0.775	0.781
c237	0.847	0.827	0.838	0.773	0.849	0.814	0.792	0.836
c361	0.789	0.781	0.792	0.764	0.797	0.795	0.734	0.784
W-1	0.811	0.792	0.797	0.748	0.803	0.784	0.729	0.806
W-2	0.781	0.806	0.784	0.762	0.762	0.781	0.704	0.764
S-2	0.806	0.775	0.792	0.748	0.775	0.767	0.707	0.767
S-7	0.847	0.855	0.844	0.762	0.795	0.792	0.759	0.797
S-47	0.806	0.836	0.814	0.808	0.803	0.806	0.745	0.816

	C46	C49	C51	C57	C60	C62	C66	C80
S-65	0.816	0.825	0.814	0.803	0.814	0.827	0.762	0.795
S-70	0.814	0.838	0.833	0.8	0.811	0.808	0.764	0.797
S-70A	0.819	0.822	0.816	0.784	0.8	0.792	0.753	0.792
S-70B	0.858	0.822	0.849	0.784	0.833	0.836	0.764	0.836
S-72	0.871	0.83	0.841	0.792	0.808	0.816	0.762	0.795
S-73	0.816	0.797	0.83	0.775	0.814	0.827	0.762	0.811
S-179	0.8	0.792	0.797	0.792	0.819	0.8	0.745	0.795
S-180	0.849	0.825	0.841	0.803	0.808	0.816	0.762	0.795
S-194	0.77	0.762	0.762	0.756	0.762	0.77	0.699	0.753
S-199	0.811	0.819	0.83	0.775	0.775	0.789	0.756	0.773
S-DB	0.759	0.767	0.795	0.74	0.773	0.775	0.71	0.781
S-M1	0.814	0.795	0.827	0.778	0.806	0.781	0.759	0.786
ABR-26	0.759	0.767	0.773	0.767	0.745	0.764	0.726	0.753
ABR-14	0.803	0.811	0.816	0.784	0.784	0.803	0.759	0.764
MG-21	0.841	0.822	0.827	0.789	0.795	0.808	0.786	0.819
MKT-161	0.786	0.789	0.767	0.778	0.789	0.797	0.775	0.775

	C86	C88	C90	C94	C103	C107	C113	C114
c86	1							
c88	0.764	1						
c90	0.819	0.83	1					
c94	0.8	0.833	0.86	1				
c103	0.8	0.822	0.855	0.896	1			
c107	0.743	0.808	0.803	0.838	0.838	1		
c113	0.734	0.8	0.811	0.83	0.858	0.86	1	
c114	0.789	0.8	0.822	0.83	0.874	0.822	0.825	1
c124	0.806	0.827	0.849	0.863	0.858	0.8	0.808	0.869
c128	0.767	0.806	0.86	0.858	0.863	0.811	0.825	0.83
c168	0.797	0.775	0.852	0.827	0.811	0.786	0.8	0.795
c169	0.751	0.784	0.806	0.808	0.814	0.795	0.781	0.797
c174	0.789	0.795	0.833	0.841	0.836	0.795	0.797	0.83
c175	0.773	0.8	0.833	0.83	0.825	0.806	0.814	0.825
c192	0.786	0.808	0.836	0.855	0.833	0.786	0.784	0.827
c198	0.767	0.778	0.795	0.797	0.83	0.806	0.792	0.808
c201	0.751	0.8	0.811	0.83	0.814	0.8	0.77	0.814
c204	0.74	0.778	0.778	0.792	0.792	0.789	0.781	0.797
c232	0.745	0.778	0.789	0.814	0.797	0.767	0.792	0.819
c235	0.732	0.743	0.786	0.8	0.795	0.797	0.778	0.789
c237	0.759	0.797	0.841	0.811	0.816	0.775	0.784	0.816
c361	0.734	0.751	0.762	0.808	0.803	0.767	0.743	0.797
W-1	0.74	0.806	0.773	0.781	0.781	0.74	0.732	0.77
W-2	0.715	0.781	0.759	0.762	0.811	0.77	0.789	0.784
S-2	0.734	0.734	0.756	0.77	0.781	0.74	0.726	0.759
S-7	0.77	0.781	0.808	0.816	0.8	0.781	0.762	0.795
S-47	0.756	0.827	0.795	0.836	0.814	0.811	0.792	0.803
S-65	0.778	0.778	0.789	0.803	0.819	0.778	0.792	0.819
S-70	0.759	0.786	0.792	0.811	0.789	0.781	0.756	0.778
S-70A	0.753	0.764	0.77	0.795	0.806	0.764	0.762	0.795
S-70B	0.764	0.803	0.814	0.833	0.833	0.792	0.767	0.816
S-72	0.756	0.8	0.789	0.83	0.852	0.806	0.803	0.819
S-73	0.767	0.789	0.811	0.814	0.825	0.795	0.775	0.803
S-179	0.729	0.795	0.778	0.808	0.808	0.767	0.759	0.792
S-180	0.756	0.789	0.8	0.803	0.814	0.773	0.759	0.803
S-194	0.732	0.792	0.748	0.74	0.74	0.732	0.734	0.74
S-199	0.751	0.74	0.767	0.786	0.797	0.773	0.775	0.781
S-DB	0.732	0.753	0.792	0.789	0.795	0.743	0.762	0.751

Continued the Appendix

	C86	C88	C90	C94	C103	C107	C113	C114
S-M1	0.753	0.775	0.786	0.795	0.8	0.77	0.789	0.789
ABR-26	0.699	0.764	0.737	0.74	0.767	0.764	0.745	0.767
ABR-14	0.737	0.775	0.775	0.8	0.822	0.781	0.795	0.8
MG-21	0.753	0.775	0.792	0.822	0.827	0.825	0.795	0.816
MKT-161	0.748	0.753	0.77	0.8	0.795	0.781	0.784	0.773
	C124	**C128**	**C168**	**C169**	**C174**	**C175**	**C192**	**C198**
c124	1							
c128	0.863	1						
c168	0.827	0.838	1					
c169	0.792	0.797	0.773	1				
c174	0.841	0.858	0.811	0.825	1			
c175	0.836	0.83	0.811	0.792	0.863	1		
c192	0.849	0.827	0.786	0.789	0.838	0.838	1	
c198	0.792	0.786	0.756	0.748	0.814	0.819	0.838	1
c201	0.819	0.825	0.745	0.797	0.841	0.836	0.888	0.814
c204	0.781	0.786	0.751	0.77	0.819	0.83	0.838	0.814
c232	0.808	0.792	0.767	0.786	0.797	0.819	0.838	0.825
c235	0.778	0.784	0.775	0.784	0.811	0.811	0.836	0.811
c237	0.849	0.822	0.803	0.778	0.833	0.833	0.863	0.827
c361	0.792	0.77	0.74	0.803	0.819	0.786	0.849	0.797
W-1	0.786	0.764	0.767	0.792	0.792	0.781	0.811	0.781
W-2	0.784	0.756	0.726	0.756	0.762	0.762	0.759	0.811
S-2	0.775	0.775	0.723	0.759	0.786	0.781	0.816	0.786
S-7	0.822	0.806	0.803	0.767	0.795	0.827	0.852	0.811
S-47	0.83	0.808	0.773	0.819	0.797	0.808	0.822	0.792
S-65	0.83	0.803	0.767	0.797	0.814	0.819	0.849	0.775
S-70	0.806	0.8	0.775	0.784	0.811	0.795	0.858	0.8
S-70A	0.784	0.767	0.748	0.773	0.816	0.784	0.814	0.806
S-70B	0.838	0.811	0.786	0.806	0.844	0.816	0.869	0.806
S-72	0.819	0.797	0.767	0.819	0.852	0.83	0.855	0.819
S-73	0.814	0.808	0.762	0.786	0.825	0.803	0.827	0.77
S-179	0.797	0.77	0.745	0.764	0.814	0.792	0.816	0.781
S-180	0.803	0.797	0.789	0.753	0.819	0.808	0.838	0.814
S-194	0.773	0.734	0.732	0.762	0.778	0.784	0.781	0.751
S-199	0.781	0.792	0.789	0.781	0.808	0.814	0.822	0.792
S-DB	0.784	0.756	0.737	0.729	0.778	0.806	0.803	0.773
S-M1	0.8	0.795	0.781	0.756	0.811	0.8	0.814	0.778
ABR-26	0.756	0.756	0.726	0.784	0.773	0.8	0.775	0.762
ABR-14	0.827	0.8	0.786	0.767	0.822	0.827	0.814	0.811
MG-21	0.8	0.784	0.781	0.789	0.816	0.844	0.83	0.833
MKT-161	0.789	0.789	0.759	0.773	0.811	0.833	0.803	0.784
	C201	**C204**	**C232**	**C235**	**C237**	**C361**	**W-1**	**W-2**
c201	1							
c204	0.847	1						
c232	0.852	0.847	1					
c235	0.838	0.849	0.844	1				
c237	0.86	0.833	0.866	0.836	1			
c361	0.814	0.814	0.814	0.816	0.811	1		
W-1	0.825	0.792	0.803	0.806	0.827	0.814	1	
W-2	0.778	0.778	0.773	0.759	0.792	0.751	0.795	1
S-2	0.819	0.77	0.77	0.762	0.8	0.781	0.759	0.778
S-7	0.844	0.811	0.822	0.83	0.858	0.778	0.816	0.786
S-47	0.836	0.808	0.819	0.827	0.827	0.797	0.814	0.795
S-65	0.858	0.825	0.836	0.833	0.827	0.825	0.819	0.795

	C201	C204	C232	C235	C237	C361	W-1	W-2
S-70	0.849	0.806	0.822	0.825	0.83	0.795	0.833	0.775
S-70A	0.822	0.827	0.816	0.803	0.825	0.795	0.811	0.781
S-70B	0.877	0.844	0.871	0.847	0.858	0.827	0.827	0.775
S-72	0.869	0.858	0.836	0.833	0.844	0.819	0.814	0.8
S-73	0.83	0.797	0.797	0.811	0.833	0.808	0.797	0.784
S-179	0.852	0.803	0.814	0.811	0.822	0.808	0.803	0.778
S-180	0.841	0.797	0.808	0.822	0.844	0.792	0.797	0.784
S-194	0.8	0.789	0.789	0.775	0.775	0.778	0.789	0.753
S-199	0.814	0.803	0.819	0.844	0.806	0.792	0.792	0.773
S-DB	0.789	0.784	0.789	0.803	0.808	0.756	0.778	0.743
S-M1	0.8	0.789	0.811	0.819	0.814	0.784	0.773	0.764
ABR-26	0.784	0.784	0.773	0.786	0.775	0.756	0.756	0.77
ABR-14	0.827	0.811	0.822	0.819	0.83	0.789	0.784	0.797
MG-21	0.822	0.827	0.838	0.841	0.836	0.806	0.795	0.786
MKT-161	0.827	0.816	0.8	0.803	0.808	0.784	0.745	0.759
	S-2	**S-7**	**S-47**	**S-65**	**S-70**	**S-70A**	**S-70B**	**S-72**
S-2	1							
S-7	0.822	1						
S-47	0.786	0.871	1					
S-65	0.814	0.833	0.863	1				
S-70	0.811	0.847	0.882	0.866	1			
S-70A	0.795	0.825	0.827	0.838	0.847	1		
S-70B	0.833	0.852	0.838	0.877	0.847	0.863	1	
S-72	0.797	0.855	0.847	0.841	0.827	0.833	0.888	1
S-73	0.792	0.827	0.803	0.841	0.8	0.822	0.866	0.858
S-179	0.775	0.806	0.814	0.847	0.833	0.816	0.855	0.841
S-180	0.808	0.838	0.814	0.841	0.827	0.838	0.871	0.83
S-194	0.762	0.775	0.778	0.811	0.797	0.759	0.83	0.806
S-199	0.764	0.822	0.797	0.814	0.811	0.795	0.844	0.841
S-DB	0.751	0.786	0.778	0.806	0.814	0.792	0.803	0.795
S-M1	0.789	0.814	0.816	0.822	0.819	0.825	0.836	0.827
ABR-26	0.784	0.803	0.795	0.767	0.786	0.764	0.792	0.806
ABR-14	0.778	0.825	0.816	0.827	0.814	0.797	0.814	0.838
MG-21	0.795	0.841	0.822	0.827	0.83	0.836	0.874	0.855
MKT-161	0.767	0.792	0.795	0.827	0.808	0.797	0.825	0.822
	S-73	**S-179**	**S-180**	**S-194**	**S-199**	**S-DB**	**S-M1**	**ABR-26**
S-73	1							
S-179	0.83	1						
S-180	0.83	0.841	1					
S-194	0.806	0.849	0.806	1				
S-199	0.803	0.83	0.88	0.806	1			
S-DB	0.806	0.811	0.806	0.77	0.822	1		
S-M1	0.811	0.849	0.849	0.803	0.844	0.847	1	
ABR-26	0.778	0.8	0.795	0.797	0.8	0.748	0.808	1
ABR-14	0.806	0.838	0.827	0.803	0.844	0.803	0.858	0.874
MG-21	0.822	0.833	0.838	0.808	0.833	0.814	0.869	0.797
MKT-161	0.795	0.816	0.806	0.775	0.811	0.77	0.808	0.77
	ABR-14	MG-21	MKT-161					
ABR-14	1							
MG-21	0.858	1						
MKT-161	0.819	0.836	1					